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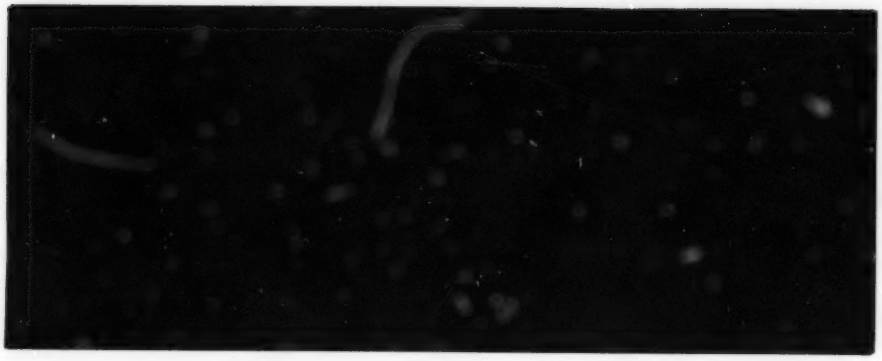

Final Report

2.4-20

The Value of Parental Copper Glycinate, Oral Sustained Release Copper Oxide and Free Choice Trace Minereal Salt in Improving the Copper Status of Beef Cows and their Calves

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FINAL REPORT:

The value of parenteral copper glycinate, oral sustained release copper oxide needles and free choice trace mineral salt in improving the copper status of beef cows and their calves. 2.4.20.

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1.0 EXECUTIVE SUMMARY

1.1 Abstract

One hundred commercial Hereford cows were divided equally into five treatments: no copper supplement, subcutaneous copper glycinate every three months, every 6 months, oral copper oxide needles or free choice copper fortified salt (0.83% actual copper). The trial started when the cows were 90 days pregnant and ended when the calves were weaned. Free choice copper fortified salt was the most effective in improving the copper status of the cow and her calf followed by the copper oxide needles. Copper glycinate was the least effective and caused a severe local tissue reaction after the first injection.

1.2 Summary

In Saskatchewan a high percentage of cattle feeds and pastures are deficient in copper (< 10 mg Cu / kg DM). Also many of the farm water supplies are high in sulphates (> 1000 mg/l). Sulphates interact with dietary copper in the rumen making it unavailable for absorption.

The results of this trial indicate that the use of a copper fortified salt is satisfactory in improving the copper status of cows. If this supplement is made available to the cows during pregnancy it will have a positive impact on the copper status of the calf. Free choice salt intake must be monitered and the level of copper supplementation adjusted according to salt intake and dietary concentrations of copper.

Copper glycinate injections are only available from a licenced veterinarian with special permission from the Food and Drug Directorate. This form of supplementation is practical when oral supplementation is not. This supplement is only effective for three months. The livestock producer runs the risk of severe local tissue reactions with subsequent injections and thus less copper is available from the site of the injection. This form of supplementation when given to pregnant cows only marginally contributes the copper reserves of the developing fetus.

Oral copper oxide needles are also only available through licenced veterinarians with special permission. This form of copper supplementation is effective in bypassing the negative effects of

sulphates and molybdenum on copper in the rumen. This supplement is also effective in improving the copper status of the fetus but must be given at approximately 3 month intervals.

2.0 TECHNICAL REPORT

2.1 Abstract

One hunderd commercial mature pregnant Hereford cows were divided equally into five treatment groups: no copper supplement, subcutaneous copper glycinate every 3 months, copper glycinate every 6 months, oral copper oxide needles or free choice copper fortified salt. The trial started on October 3, 1987 and ended when the calves were weaned on August 30, 1988. Blood and liver biopsies were taken for copper analysis from the cows and their calves at specific times during the trial. The subcutaneous copper glycinate injections caused a severe local tissue reaction after the first injection. The specially formulated trace mineralized salt (0.83% actual copper) was the most effective in improving the copper status of the cow and her calf. During the winter feeding period the cows consumed 50 to 60 gms of salt per head per day. Copper oxide needles were the next effective, but the effect on the cow did not last for the duration of the trial and was most effective over the first 90 days. The copper glycinate was the least effective and had to be given at approximatly three months intervals which increased the risk of a severe local reaction.

2.2 Background and Objectives

Primary (a dietary lack) and secondary (dietary interactions) copper deficiencies are common in Western Canada. A high percentage of Saskatchewan forages and grains fed to cattle are deficient in copper (< 10 mg/kgDM). Saskatchewan deep well waters are often high

in sulphates >1000mg/l. Sulphates interact alone or with molybdenum in the rumen to depress the availability of dietary copper. The Tremuende Reasearch Farm offers a unique opportunity to study copper deficiency in cattle under practical farm conditions, as the feedstuffs are naturally deficient in copper and the deep well water is high in sulphates.

Copper supplementation of the pregnant beef cow is essential if the calf is to be born with adequate liver copper reserves. Supplementation of the dam is critical during the last trimester of pregnancy. If the cow's diet is deficient at this time the calf will be born with deficient copper reserves. The calf's copper status to weaning is dependent on its liver copper reserves at birth.

We have studied several means of overcoming the inhibitory effects of high sulphates on copper availability. Sulphate removal from the water by reverse osmosis is effective but very expensive. The use of a subcutaneous injection of copper EDTA (Coprin, Glaxco) is beneficial. To be effective this product must be used every 3 months and research indicates that the effectiveness of this product decreases with prolonged use. Research into the use of oral copper oxide needles suggest that this form of supplementation is effective as the needles are slowly broken down in the abomasum.

The only means of supplementation readily available to the producer are trace mineral fortified mineral or salt supplements that can be fed free choice or mixed in a prepared ration. If the formulation of these products is correct and the intake is adequate

these products offer a cheap and simple means of improving the copper status of cattle. No published research is available on the effectiveness of these supplements.

The objective of this research was to determine the value of a free choice specially formulated trace mineralized salt, of parenteral copper glycinate (Moly Cu, Schering) not yet approved for use in Canada, of oral copper oxide needles (Cooporal, Beecham Pharm) in improving the copper status of pregnant beef cows. This project will also determine the extent of placental transfer of these supplements.

2.3 Experimental Methods

Experimental Groups:

One hundred mature commercial Hereford cows were randomly assigned to 5 treatment groups (20 cows per group):

Cu 0 - No supplemental copper

Moly Cu 3 - Injectable copper glycinate every 3 months

Moly Cu 6 - Injectable copper glycinate every 6 months

Cu Ox - Oral sustained release copper oxide needles

Cu Salt - Free choice copper fortified salt

The cows were bred to calve mid-March through April. The trial was started on October 7,1987 (day 0) and ended on August 30, 1988 (day 328). At the start of the trial the cows were 80 to 90 days pregnant. The cows were confined to the corrals until after calving. On June 14,1988 (day 251) the cows were put on pasture. The Cu Salt group were maintained on a separate pasture.

Rations:

Over the winter period the cattle were fed an average of 30 kg of barley silage per head per day. (Table 1) All groups were fed free choice, a specially formulated trace mineral salt (Sifto Salt, Domtar,Ontario) with no copper added. The Cu Salt group had 0.83% actual copper added to the salt. The initial formulation of the salt was based on an estimated daily salt intake of 30 gm/head/day. The salt contained 0.2% actual manganese, 0.5% actual zinc, 0.025% actual selenium and a vitamin A, D, E premix. The trace minerals were supplied in the oxide form.

The water supply during the dry-lot period was from a deep well and contained approximately 508 mg sulphur/L. On pasture the water supply was from dugouts which were not high in sulphur.

Copper Glycinate Injections:

Twenty cows were injected subcutaneously with copper glycinate (Moly Cu. Schering, New Jersey) on Oct 7/87, Jan 6/88 and June 14/88. Another group of 20 cows was injected subcutaneously with copper glycinate on Oct 7/87 and June 14/88. Each injection contained 120 mg of actual copper.

Oral Copper Oxide Needles:

On Oct 7/87 twenty cows were given orally one gelatin capsule containing copper oxide needles (Cooporal, Beecham Pharm. New Jersey). Each capsule supplied 24 g of actual copper.

Blood and Liver Collection and Analysis:

Blood samples were taken from the jugular vein into special heparinized tubes for plasma copper and zinc determinations (B-D

Vacutainer Brand, Ingram and Bell, Don Mills, Ontario). The blood samples were centrifuged and the plasma stored at -20°C. Blood samples were taken from the cows on Oct 7/87, Jan 6/88, at calving, June 14/88 and Aug 30./88 and from the calves at birth, on June 14/88 and Aug 30/88.

Percutaneous liver biopsies were taken from the cows on Oct 7/87, Jan 6/88, June 14/88 and Aug 30/88. The calves were biopsied on June 14/88 and Aug 30/88.

Prior to chemical analysis the liver biopsies were freeze dried at -38°C and the plasma deproteinized. After freeze drying the liver samples were digested by rapid nitric perchloric acid. The liver and plasma samples were then analysed for copper, zinc and iron on a Perkin Elmer Atomic Absorption Spectrophotometer Model 5000.

Cow and Calf Body Weights:

At each sample time the cows and calves were weighted on an electronic scale. The average daily gain between each period was calculated and an adjusted weaning weight was determined for the calves.

Statistical Analysis:

The data were analysed by an analysis of variance using the general linear model (GLM), a procedure described by the Statistical Analysis System for unbalanced data. Least square means were computed and these means were compared by an option allowed in the GLM procedure. The level of significance for this report is $p<0.05$.

2.4 RESULTS

Copper Glycinate Injections:

Four days after the June 14th subcutaneous injection of copper glycinate all the cows experienced a severe local tissue reaction. This reaction was most severe in the cows that had received the injection for the third time. The reaction associated with the injection was a severe edema of the brisket. This swelling was painful and many of the cows were reluctant to move and graze. Despite the severe tissue reaction none of these cows had an elevated temperature. The reaction lasted for a period of 4 to 5 days. On August 30/88 a few cows still had small lumps on their briskets, but there appeared to be no long-term severe effects.

Free Choice Salt Intake

The free choice salt intake per pen was quite variable over the dry-lot period. The average salt consumption for the non copper fortified salt groups was 50.5±9.7 gm/head/day. The copper fortified salt was consumed at 65.5±3.3 gm/head/day. Although the cows were fed the salt throughout the trial, salt consumption was only monitored over the winter period, as other cattle were on the pastures with the trial cattle. Free choice salt intake was influenced by the pen location and the location of the salt within the pen. Salt consumption was highest for the cattle in the pen located near the area of most activity. Salt consumption did not appear to be influenced by copper supplementation.

Trace Mineral Intake

The trace mineral content of the salt was based on an assumed consumption of 30 gm/head/day. This level of consumption was based on previous trials carried out at the research farm. In this trial the salt intake was in excess of the assumed amount.

The estimated individual daily copper intake is presented on Table 2. All estimates are approximate, individual intake values are based on estimates obtained from group intakes. The true availability of dietary copper .03 (3%) was calculated from previous trials and based on the interactions of dietary sulphur and molybdenum.

From the results on Table 2, the copper fortified salt theoretically supplied the cow with the most available copper. This copper would be available to support copper dependent biochemical functions, to supply the developing fetus, to maintain plasma copper and to add to liver copper reserves.

The copper oxide needles in practice may supply more available copper than calculated. The copper oxide needles are retained in the abomasum and thus bypass the interactions in the rumen between copper and molybdenum/sulphur. Thus more copper may be available for digestion in the upper small intestine.

The calculations for the injectable copper glycinate are based on an assumed constant daily release of copper from the site of subcutaneous injection or from the liver were the copper is rapidly stored. As a result of the severe local tissue reactions that occurred after the initial injection this is likely not accurate.

The total daily zinc intake per cow was 250 mg for the non copper fortfied salt groups and 330 mg for the copper fortified salt group. The total daily iron intake came only from the silage and was 4657 mg/day for all treatment groups.

Cow Plasma and Liver Copper Concentrations (Tables 3 & 4 ; Figures 1 & 2)

A liver copper concentration of less than 0.55 umol/gm DM is indicative of a deficient copper status. Plasma copper concentrations of less than 11.0 umol/l are indicative of a marginal to deficient copper status.

At the start of the trial, all treatment groups except the Cu Salt group had a deficient plasma copper. In the Cu O group the plasma copper concentration declined significantly to calving. After calving there was a gradual improvement in the plasma copper. By day 90 the plasma copper had increased in all the other treatment groups, with the Cu Ox group having the greatest improvement. At calving, the plasma copper had declined in all groups, with the most significant decline occurring in the Moly Cu 6 and 3 groups. By day 251 the plasma copper in the Moly Cu 3 and the Cu Ox groups had declined further. In the Moly Cu 6 and the Cu Salt groups there was an improvement in the plasma copper concentrations. By the end of the trial, the plasma copper concentrations had improved in all treatment groups and were indicative of an adequate copper status.

At the start of the trial, the liver copper concentrations in all treatment groups were deficient. By Day 90, the liver copper in the Cu0 group had declined, all other treatment groups had increased. With the most significant increase in the Cu Salt and Cu Ox groups. By day 251, the liver copper concentrations in all groups had declined significantly, with the Cu Salt group having a

significantly higher liver copper concentration than all the other groups. Over the pasture period, the liver copper concentrations improved over all treatments, with a significant improvement occurring in the Moly Cu treatment group.

Although the copper oxide needles resulted in the greatest improvement in the liver copper concentrations initially, the copper fortified salt resulted in a more sustained improvement in the copper status over the trial period. The daily copper intake although adequate in maintaining plasma copper concentration was not adequate in maintaining liver copper concentrations.

Table 7 illustrates the percentage of the available dietary copper in the liver for each treatment group after 90 days of treatment. A high percentage of copper from the copper oxide needles was available for storage in the liver supporting the theory that the needles are released in the abomasum and bypass the interractions that occur in the rumen, thus underestimating the available copper.

Although there was a large amount of available copper in the Cu Salt group only a small percentage appeared in the liver. In this case the interactions occurring in the rumen may have been underestimated and the available copper over estimated.

Calf Liver and Plasma Copper Concentration (Table 5 & 6 ; Figures 3 & 4)

Liver copper concentration of a calf up to weaning is a reflection of the copper status of the dam during pregnancy and the degree of placental transfer of copper. If the dam is copper

deficient the liver copper reserves of the fetus start to decline after 180 days of gestation, if the dam is supplemented with copper then the liver copper reserves continue to increase. At approximately 180 days of gestation (day 91) all copper supplements should be having a positive effect on the fetus's liver copper reserves.

All dam copper treatments resulted in a significant improvement in calf liver copper concentrations except for the Moly Cu 6 group. The most significant improvement occurred in the calves whose dam's were supplemented with the copper fortified salt. Over the pasture period there was a decline in the liver copper of all calves, but the copper fortified salt calves maintained their advantage. This indicates that some of the supplemental copper passed through the placental barrier and was stored in the fetal liver. The calves were biopsied at 3 to 4 months of age because biopsies prior to this time may be too stressful.

The initial low plasma copper concentration at birth is a normal physiological occurrence and is not a reflection of a deficient copper status. The sustained low plasma copper concentration in the Cu 0 calves is a reflection of a deficient copper status. The plasma copper concentrations of the calves at weaning reflected the liver copper concentrations at that time. Although all but the Cu Salt had deficient liver copper concentrations only the plasma concentration of the Cu0 calves reflected a deficient status. This illustrates the weakness of plasma copper concentrations as a reflection of copper status.

Cow plasma and liver zinc concentrations (Tables 8 & 9)

Plasma zinc concentrations indicative of an adequate status are 10.8 to 21.4 umol/l. Plasma zinc concentrations are not only affected by dietary intake but also decline rapidly in response to stress and the acute phase inflammatory response. The dietary zinc intake for this trial was considered adequate, thus any changes in plasma zinc would be physiological not dietary. This point is illustrated by the decline in plasma zinc that occurred at calving which is a stressful time.

Adequate liver zinc concentrations are 0.51 to 2.6 umol/gm DM. The liver zinc concentrations for all groups reflected an adequate dietary intake.

Calf plasma and liver zinc concentrations (Tables 10 & 11)

Calf plasma zinc concentrations at birth were twice that of their dams. This is a normal physiological response. The significant differences seen at birth between treatment groups was not related to the copper treatment, but that the blood samples were not taken at the same time after birth for each calf. After birth plasma zinc concentrations fluctuate significantly and stabilize after 4 days. At subsequent sample periods the plasma zinc concentrations remained in the normal range.

Calf liver zinc concentration was not infuenced by copper treatment and remained in an adequate range throughout the trial.

Cow plasma and liver iron concentrations (Tables 12 & 13)

Little information is in the literature concerning normal

plasma and liver iron concentrations. Iron status is considered adequate if serum values are 8.9 to 53.7 umol/l and liver concentrations are 2.8 to 18.5 umol/gm DM. Copper deficiency results in a failure of iron being released from the storage sites in the body because of a lack of caeruloplasmin, a copper dependent enzyme.

The plasma iron concentrations through the trial were normal. Although there were significant differences in plasma iron during the trial, these were not considered to be the result of copper treatment.

Liver iron concentrations were considered normal. Conclusions on the effect of copper supplementaion on iron status are not clear from the results of this trial.

Calf plasma and liver iron concentrations (Tables 13 & 14)

Calf plasma and liver iron concentration were in an adequate range. Copper supplementation did not appear to influence plasma iron concentrations . The liver iron concentrations were significantly higher in the calves from dams not supplemented with copper indicating that liver iron stores were increased in this group.

Cow and Calf Body Weight Changes:

Copper supplementation had no effect on cow body weight, cow average daily gain, calf average daily gain or adjusted weaning weight.

Conclusions:

1) Free choice salt intake is variable but ranges from 50 to

65 gm/head/day. Free choice intake is dependent on the location of the salt in the pen.

2) Injectable copper glycinate may be a useful supplement if used as a single treatment. In this trial subsequent injections resulted in a severe local tissue reaction and an apparent reduced copper availability to the dam and fetus.

3) Oral copper oxide needles would be a valuable copper supplement, especially in situations where the use of free choice or force fed copper supplemented salt or mineral is not possible. Copper oxide needles may be a useful supplement where water or feeds are high in sulphur and/or molybdenum. The effectivness of the needles in maintaining the copper status of the cow lasts for approximately 3 months.

4) Free choice copper supplemented salt appears to be a satisfactory means of copper supplementation. Where dietary interactions occur it may be necessary to add concentrations of copper above those presently available commercially (> 0.5% actual copper). Under the conditions of this trial free choice supplementation on the pastures was not adequate to maintain the copper status.

5) Copper supplementation of the dam during pregnancy is essential if the calf is to be born with an adequate liver copper reserve. This is true in situations where the dietary copper intake is low (< 10 mg Cu/kgDM) or

where dietary interactions occur. Copper fortified salt was the most satisfactory supplement in improving the copper status of the fetus, followed by copper oxide needles and then copper glycinate injections every 3 months.

Impact on Saskatchewan Agriculture:

In Saskatchewan a high percentage of cattle feeds and pastures were deficient in copper < 10 mg Cu/kg DM). Also many of the farm water supplies are high in sulphates > 1000 mg/l. Sulphates interact with dietary copper in the rumen making it unavailable for absorption.

The results of this trial indicate that the use of a copper fortified salt is satisfactory in improving the copper status of cows. If this supplement is made available to the cows during pregnancy it will have a positive impact on the copper status of the calf. Free choice salt intake must be monitored and the level of copper supplementation adjusted according to salt intake and dietary concentrations of copper.

Copper glycinate injections are only available from a licensed veterinarian with special permission from the Food and Drug Directorate. This form of supplementation is practical when oral supplementation is not. This supplement is only effective for three months. The livestock producer runs the risk of severe local tissue reactions with subsequent injections and thus less copper is available from the site of the injection. This form of supplementation when given to pregnant cows only marginally contribute the copper reserves of the developing fetus.

Oral copper oxide needles are also only available through licensed veterinarians with special permission. This form of copper supplementation is effective in bypassing the negative effects of sulphates and molybdenum on copper in the rumen. This

supplement is also effective in improving the copper status of the fetus but must be given at approximately 3 month intervals.

3.0 TECHNOLOGY TRANSFERS:

To date no formal papers or talks have been presented. The results of this trial have been sent to the Food and Drug Directorate to alert them to the adverse reactions to the copper glycinate injections.

A paper has been prepared for publication in the Canadian Veterinary Journal. This paper is a review of all the research on copper metabolism in cattle that has been carried out in Canada since 1950. This paper includes recommendations for supplementation and thus some of the results from this trial. In discussing copper supplementation in my lectures to veterinary students, I use the results of this trial for my recommendations.

The results of this trial have also been published in the 1990 Termuende Research Reports and presented at the annual field day in June,1990.

4.0 PERSONNEL:

1) Elaine Dribnenky: Technical assistant, 100% of her time was devoted to this project

2) Dr. M.E. Smart: Reasearch coordinator, 20% of time devoted to this project.

3) Dr. L. Petrie and Dr. J. Robinson assisted in sample collection.

4.1 Status of the Graduate Student

A graduate student could not be found in time to conduct this trial. The money for the student has been retained in the account.

5.0 EQUIPMENT

No equipment for a value of $500 or more was purchased for this project.

Table 1 : Feed Analysis of Barley Silage (as fed)

Moisture %	Protein %	TDN %	Ca %	P %	Mg %	Mn ppm	Cu ppm	Zn ppm
77	3.7	13	.12	.06	.06	8.5	1.2	7.2

Table 2. Estimated individual total daily and available copper intake for each copper supplementation group during the drylot period.

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
daily salt intake (g)	50.5	50.5	50.5	50.5	65.5
daily silage intake (kg)	30.0	30.0	30.0	30.0	30.0
Cu from silage (mg/d)	36.9	36.9	36.9	36.9	36.9
Cu from glycinate (mg/d)	–	1.3	0.7	–	–
Cu from Cu oxide needle (mg/d)	–	–	–	64.6	–
total dietary Cu (mg/d)	36.9	36.9	36.9	101.5	580.0
mg Cu/kg DM	5.7	5.7	5.7	14.5	83.0
total available Cu (mg/d)	1.1	2.4 ¥	1.8 ¥	3.1	17.4

† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.
¥ Assume all Cu from subcutaneous injections is available.

Table 3. Cow plasma copper concentration (μmol/L)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 0 Oct. 3/87	10.76^{fghi}	10.52^{ghi}	9.79^{ij}	10.20^{hi}	12.49^{def}
Day 91 Jan. 6/88	5.67^{l}	12.95^{cde}	13.39^{bcde}	14.08^{abcd}	11.96^{efgh}
calving	2.55^{n}	9.77^{ij}	3.65^{m}	12.85^{cde}	11.53^{efghi}
Day 251 June 14/88	5.98^{l}	6.87^{kl}	5.27^{lm}	7.99^{k}	13.54^{bcde}
Day 328 Aug. 30/88	12.30^{efg}	15.32^{ab}	15.77^{a}	12.44^{def}	14.61^{abc}

Pooled standard error of the means = ±0.70.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 4. Cow liver copper concentration (μmol/g DM)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 0 Oct. 3/87	0.48^{cdefg}	0.38^{defghi}	0.26^{efghij}	0.34^{efghij}	0.49^{cdef}
Day 91 Jan. 6/88	0.22^{ghij}	0.50^{cde}	0.43^{cdefgh}	2.44^{a}	1.05^{b}
calving	–	–	–	–	–
Day 251 June 14/88	0.11^{ij}	0.09^{j}	0.08^{j}	0.09^{j}	0.43^{cdefgh}
Day 328 Aug. 30/88	0.15^{hij}	0.64^{cd}	0.51^{cde}	0.23^{fghij}	0.66^{c}

Pooled standard error of the means = ±0.10.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

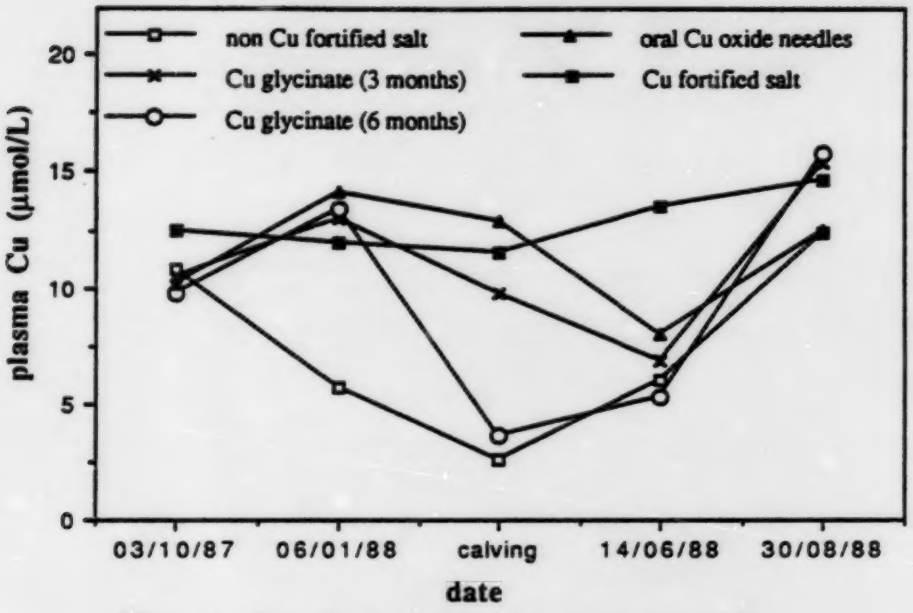

Figure 1. Cow plasma copper concentration (µmol/L).

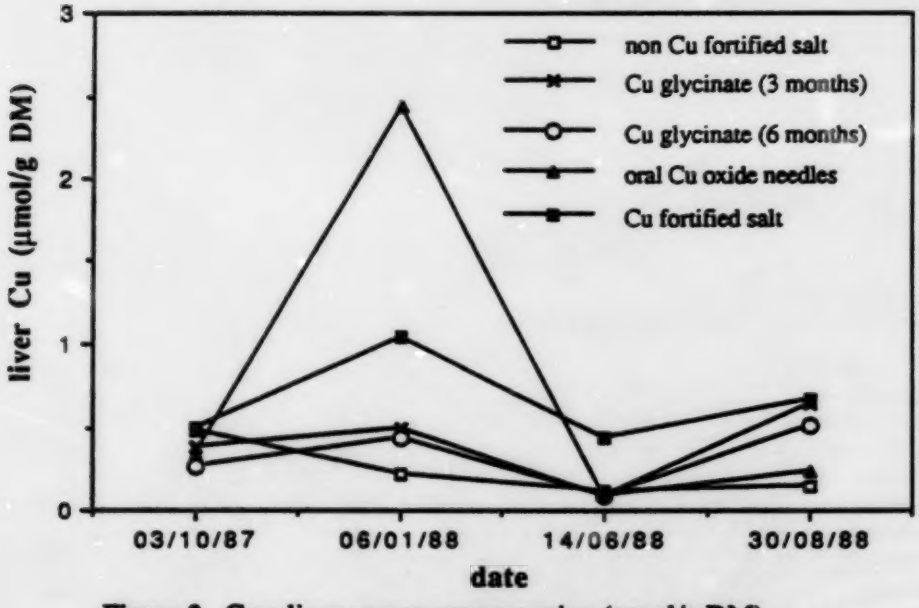

Figure 2. Cow liver copper concentration (µmol/g DM)

Table 5. Calf plasma copper concentration (µmol/L)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
birth	5.37^{hi}	6.25^{ghi}	5.27^{i}	5.22^{i}	7.23^{gh}
Day 251 June 14/88	5.13^{i}	12.33^{bcd}	8.02^{fg}	13.14^{abc}	11.62^{cde}
Day 328 Aug. 30/88	9.88^{ef}	11.66^{cde}	11.08^{de}	13.54^{ab}	14.45^{a}

Pooled standard error of the means = ±0.67.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 6. Calf liver copper concentration (µmol/g DM)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 251 June 14/88	0.18^{e}	0.77^{cd}	0.31^{e}	0.88^{c}	2.36^{a}
Day 328 Aug. 30/88	0.13^{f}	0.37^{e}	0.21^{e}	0.47^{d}	1.62^{b}

Pooled standard error of the means = ±0.14.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

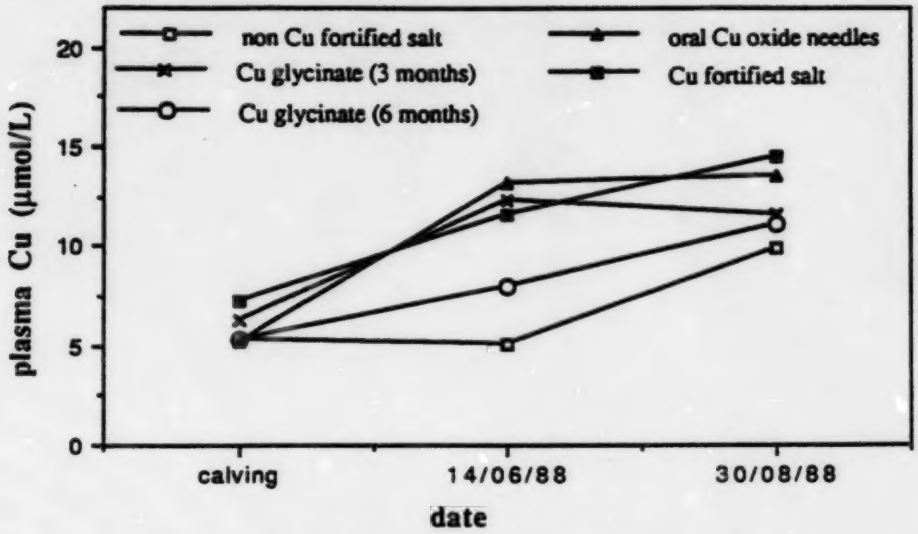

Figure 3. Calf plasma copper concentration(µmol/L)

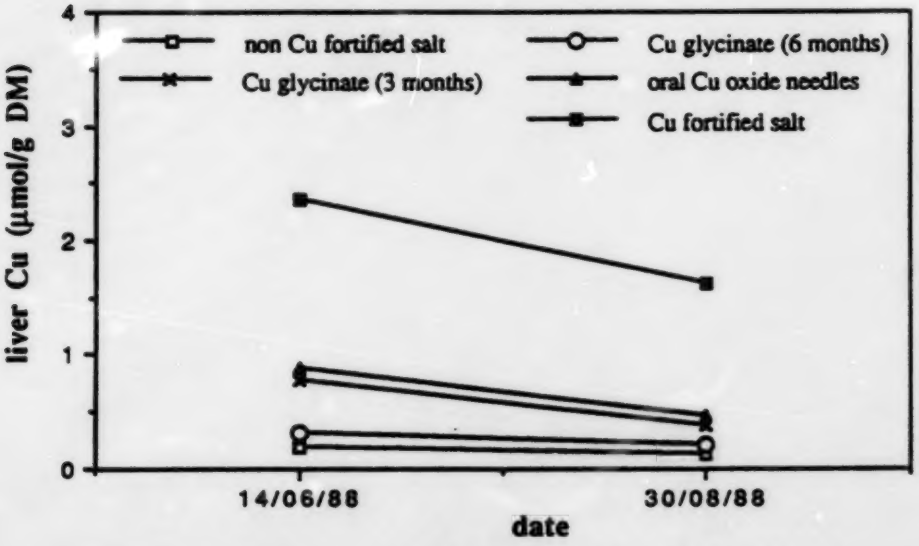

Figure 4. Calf liver copper concentration (µmol/g DM)

Table 7. Percent of daily available copper in the liver of cows after 90 days of various copper supplements.

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	Cu oxide needles §	Cu fortified salt ††
total liver Cu (mg)*					
day 0	51.9	41.8	28.1	36.7	52.9
day 90	23.8	54.1	46.4	263.5	113.4
daily change in liver Cu (mg/d)	-0.31	0.14	0.20	2.5	0.67
daily available dietary Cu (mg/d)	1.1	2.4	2.4	3.1	17.4
% of daily available Cu in liver	0	5.8	8.3	81.0	3.9

† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.
* See Appendix A.

Table 8. Cow plasma zinc concentration (μmol/L)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 0 Oct. 3/87	13.02^{efgh}	12.74^{efgh}	13.40^{efg}	12.12^{gh}	13.08^{efgh}
Day 91 Jan. 6/88	18.43^{a}	16.31^{bc}	16.94^{b}	16.68^{b}	15.22^{cd}
calving	13.59^{ef}	9.71^{k}	11.79^{hij}	10.05^{jk}	10.56^{ijk}
Day 251 June 14/88	11.96^{ghi}	11.94^{hij}	12.23^{fgh}	12.33^{fgh}	13.90^{de}
Day 328 Aug. 30/88	14.18^{de}	12.31^{fgh}	13.08^{efgh}	12.96^{efgh}	13.46^{efg}

Pooled standard error of the means = ±0.53.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 9. Cow liver zinc concentration (μmol/g DM)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 0 Oct. 3/87	1.92	1.66	1.78	1.76	1.83
Day 91 Jan. 6/88	1.66	1.61	1.51	1.51	1.65
calving	–	–	–	–	–
Day 251 June 14/88	1.57	1.46	1.47	1.47	1.53
Day 328 Aug. 30/88	1.58	1.58	1.60	1.55	1.45

Pooled standard error of the means = ±0.06.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 10. Calf plasma zinc concentration (μmol/L)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
birth	22.09^{a}	18.86^{bc}	18.62^{c}	21.25^{ab}	17.90^{cd}
Day 251 June 14/88	14.90^{e}	14.28^{e}	14.74^{e}	14.59^{e}	14.53^{e}
Day 328 Aug. 30/88	15.34d^{e}	14.07^{e}	14.42^{e}	14.13^{e}	14.63^{de}

Pooled standard error of the means = ±0.96.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 11. Calf liver zinc concentration (μmol/g DM)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 251 June 14/88	1.68^{ab}	1.68^{ab}	1.48^{b}	1.78^{ab}	1.45^{b}
Day 328 Aug. 30/88	1.85^{ab}	1.74^{ab}	2.00^{a}	2.01^{a}	1.77^{ab}

Pooled standard error of the means = ±0.15.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 12. Cow plasma iron concentration (μmol/L)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 0 Oct. 3/87	29.44^{def}	32.20^{bcde}	32.25^{bcde}	31.94^{bcde}	30.50^{cdef}
Day 91 Jan. 6/88	32.69^{bcde}	32.82^{bcd}	32.85^{bcd}	35.08^{ab}	32.69^{bcde}
calving	–	–	–	–	–
Day 251 June 14/88	30.48^{cdef}	29.43^{ef}	32.43^{bcde}	30.83^{cdef}	28.09^{f}
Day 328 Aug. 30/88	37.52^{a}	33.88^{abc}	36.84^{a}	37.38^{a}	33.10^{bc}

Pooled standard error of the means = ±1.25.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.
¥ assume all Cu from subcutaneous injections is available

Table 13. Cow liver iron concentration (μmol/g DM)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 0 Oct. 3/87	4.20^{g}	4.07^{g}	4.56^{defg}	5.26^{cde}	4.49^{defg}
Day 91 Jan. 6/88	7.17^{a}	6.19^{b}	7.01^{a}	7.16^{a}	6.40^{a}
calving	–	–	–	–	–
Day 251 June 14/88	5.39^{bcd}	3.85^{g}	5.13^{cdef}	4.25^{fg}	3.94^{g}
Day 328 Aug. 30/88	4.44^{defg}	4.38^{efg}	4.08^{g}	4.60^{cdefg}	4.27^{fg}

Pooled standard error of the means = ±0.32.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 14. Calf plasma Iron Concentration (μmol/L)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 251 June 14/88	39.83^{ab}	39.25^{ab}	44.69^{a}	40.27^{ab}	28.65^{d}
Day 328 Aug. 30/88	29.90^{cd}	32.78^{cd}	35.68^{bc}	31.58^{cd}	36.21^{bc}

Pooled standard error of the means = ±2.32.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

Table 15. Calf liver iron concentration (μmol/g DM)

	no Cu supplement	Cu glycinate (3 months) †	Cu glycinate (6 months) ‡	oral Cu oxide needles §	Cu fortified salt ††
Day 251 June 14/88	8.71^{a}	4.11^{b}	4.40^{b}	5.81^{b}	5.14^{b}
Day 328 Aug. 30/88	4.97^{b}	3.79^{b}	4.00^{b}	4.15^{b}	4.33^{b}

Pooled standard error of the means = ±0.94.
† Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7, Jan. 6, June 14.
‡ Moly Cu (Schering, New Jersey) 120mg actual Cu subcutaneous on Oct. 7 and June 14.
§ Cooperal (Beecham Pharm. England) orally on Oct. 7.
†† 0.83% actual Cu as copper oxide, free choice.

APPENDIX A: CALCULATION OF THE DAILY CHANGES IN LIVER COPPER CONCENTRATIONS (mg/day)

A) The total wet liver weight was calculated based on

% of body weight = 1.5% (Sisson & Grossman, 1953) (yrlgs)
= 1.0% (adult cattle)

Total liver weight (gm) {.015 x *body weight (kg)} x 1000

* Body weight based on the average body weight for each treatment group.

B) Total liver dry matter was calculated based on the mean dry matter content of the liver samples submitted to the laboratory.

29.3% $\pm$ .03 SE (n = 50)

Total liver dry matter (gm) = .293 x total liver wt (gm)

C) The total liver copper was calculated by using the laboratory value obtained mgCu/kg liver DM.

Total liver copper (mg) =(lab liver copper value

$$(mg/kgDM) \times \frac{\text{(total liver DM)}}{\left(\frac{1000}{}\right)\ kg}$$

D) To calculate the daily change in liver copper concentrations between liver sampling dates (D_1D_2):

$$\frac{\text{Total liver } CuD_2 (mg) - \text{Total liver } CuD_1 (mg)}{\text{Total number of days between sampling}} = \text{daily changes in liver copper concentrations (mg)}$$

This method produces similar results to those obtained by McDonald et al (1979) using the formula:

total liver DM = .300 ($\pm$.073) + .0033 ($\pm$.0003) W

W = live weight (kg)